18. (**Amended**) ~~A~~ The method ~~as in~~ of claim 1, wherein said morphogen induces a cascade of tissue-specific morphogenesis culminating in the formation of functional mammalian myocardium; and comprises a pair of folded polypeptides, the amino acid sequence of each of which comprises a sequence having at least 70% amino acid sequence homology with the C-terminal seven-cysteine domain of human OP-1, mouse OP-1, human OP-2 or mouse OP-2, residues 38-139 of SEQ ID NOs. 5, 6, 7 or 8, respectively.

19. (**Amended**) ~~A~~ The method ~~as in~~ of claim 1, wherein said morphogen is ~~selected from the group consisting of~~ OP- 1, CBMP-2A (BMP-2), ~~and~~ or CBMP-2B (BMP-4).

24. (**Amended**) A method of culturing mammalian myogenic precursor cells, comprising isolating said myogenic precursor cells, and culturing said myogenic precursor cells in a medium comprising an amount of a morphogen sufficient to promote proliferation or differentiation of said myogenic precursor cells into functional myocardium in a morphogenically permissive environment.

28. (**Amended**) A method of inducing myogenic precursor cells, naturally competent to differentiate into skeletal or smooth muscle, to differentiate into cardio myocytes, said method comprising: ~~the steps of~~ (a) contacting said myogenic precursor cells with a morphogen; and (b) maintaining the product of (a) in an environment morphogenically permissive for cardiomyogenesis.

29. (**Amended**) A method of producing replacement cardiomyocytes in a mammal in need thereof, said method comprising ~~the step of~~ implanting into said mammal myogenic precursor cells induced by the method of claim 28.

REMARKS

Claims 1-30 are pending in the application. Among them, claims 2-4, 21-23, 25-27, and 30 are withdrawn from consideration as being drawn to non-elected inventions. Applicants will cancel these claims upon indication of allowable subject matter. Claims 1, 5-20, 24, 28 and 29 are currently under consideration.

The Examiner has acknowledged that the IDS filed Jan. 8, 2002 (Paper No. 11) has been fully considered to the merits. The Examiner has also acknowledged that the amendments filed on Jan. 16, 2002 (Paper No. 12) have been entered in full.

Applicants have amended claims 5-19, 24, 28, and 29 to correct obvious typographical and/or grammatical errors, or to clarify the subject matter claimed. Applicants submit that there is no narrowing of scope in any respect due to these amendments.

Applicants respectfully request reconsideration in view of the following remarks. Issues raised by the Examiner will be addressed below in the order they appear in the Office Action.

Priority Claim

The Office Action states that a claim to priority under 35 U.S.C. 371 must contain a specific reference to such in the first paragraph of the first page of the specification. Accordingly, Applicants have amended the specification to correct this defect. Reconsideration and withdrawal of this objection is respectfully requested.

Applicants also submit that the instant application is the national stage application of an international application, filed on Dec. 19, 1997, which is before November 29, 2000. Therefore, neither a petition nor a surcharge under 37 C.F.R. 1.17(t) is required for the priority claim.

Missing Abstract

The Office Action asserts that the application does not contain an abstract of the disclosure as required by 37 C.F.R. 1.72(b).

Applicants submit that the original PCT application, published as WO 98/27995, contains an abstract on bottom of the first page. In addition, page 94 of the specification as originally filed also contains an "abstract of the Disclosure" on a separate page. Therefore, Applicants respectfully request reconsideration and withdrawal of this objection.

Objection to Specification

The Office Action objected to a few formality issues in the specification. Accordingly, Applicants have amended the specification to correct these defects. Reconsideration and withdrawal of these rejections are respectfully requested.

Claim rejections under 35 U.S.C. 102(b)

Claims 1, 5, 8-10, 15, 16, 20, 24, 28 and 29 are rejected under 35 U.S.C. 102(b) as being anticipated by Field, WO 95/14079 ("Field").

Specifically, the Office Action alleges that Field teaches a method of implanting a preparation of skeletal myoblasts as graft into myocardial tissues; and that since the graft can express morphogens, the grafted cells are treated with morphogens during and after implantation (page 9, line 31 to page 10, line 1-11 of Field).

Applicants submit that claim 1 and its dependent claims 5, 8-10, and 16 relate to a method of therapy for a mammal at risk of, or afflicted with, loss of or damage to myocardium. The method comprises at least two parts, namely (but not necessarily in that order) implanting a preparation of myogenic precursor cells into said mammal (thereafter referred to as "implanting"), and treating said myogenic precursor cells with an amount of a morphogen sufficient to promote proliferation or differentiation of said myogenic precursor cells into functional myocardium (thereafter referred to as "treating"). Therefore, to anticipate claim 1 and its dependent claims, both implanting and treating must be present in a cited reference.

Although Field may have described a few ways of *implanting* certain cells (some of which may fall within the definition of "myogenic precursor cells" as defined in the specification) into myocardial tissues of certain animals, it is completely silent about *treating* myogenic precursor cells with any morphogen to promote proliferation or differentiation of said precursor cells into functional myocardium. The only recitation of TGF-beta1, which is not a morphogen of the claimed invention in the first place (see argument below), also does not relate to the proliferation or differentiation of myogenic precursor cells into myocardium after morphogen treatment.

Although it may be true that the method described by Field can be adapted to express any protein, including morphogens of the claimed invention, in myocardial tissues implanted with the grafts, it does not necessarily follow that these grafts must express morphogens, and that the implanted grafts must be treated during and after implantation. This is analogous to saying that Mr. X could go see a movie last Saturday since he had some free time that day, so he must have gone to see a movie last Saturday. Clearly, this is not necessarily the case since Mr. X could have been fishing, hunting, cooking, reading books, or doing any other activity he might prefer. In fact, Field does not even mention the term "morphogen" once in the whole application, let alone the potential effects or morphogens on any kind of cells in any kind of biological activities. The method of Field is really a potential means of delivering any proteins to myocardial tissues via grafts, as the title of Example 5 ("Delivery of Protein via Graft") indicates. TGF-beta1, as a "well known angiogenic factor" (see page 31, line 19 of Field), was merely used as an example of the myriad of proteins that can be potentially delivered in a similar fashion. Page 9, line 31 to page 10, lines 1-11 of Field similarly recited a number of other therapeutic proteins, such as other angiogenic factors aFGF, VEGF, and HGF useful for induction of neovascularization, and other neurotrophic agents for ameliorating arrhythmogenesis. Apparently, none of these proteins are morphogens, and none of these proteins have anything to do with proliferation or differentiation of myogenic precursor cells into functional myocardium.

The Office Action alleges that "[t]he specification defines morphogens (growth factors) as proteins regulating cell proliferation and/or differentiation (page 3, lines 23-25)." Applicants submit that this is misguided. The quoted passage reads: "A great many proteins have now been identified which appear to act as morphogenetic or growth factors, regulating cell proliferation and/or differentiation. Typically these growth factors exert their effects on specific subsets of cells and/or tissues. Thus, for example, epidermal growth factors, nerve growth factors, fibroblast growth factors, various hormones, and many other proteins inducing or inhibiting cell proliferation or differentiation have been identified and shown to affect some subset of cells or tissues." However, the paragraph immediately following the above passage reads: "One group of morphogenetic proteins, referred to herein as "morphogens," includes members of the family of bone morphogenetic proteins (BMPs) which were initially identified by their ability to induce ectopic, endochondral bone morphogenesis. Subsequent characterization of the nucleic acid and

amino acid sequences of the BMPs has shown them to be a subgroup of the TGFβ superfamily of growth and differentiation factors."

Therefore, "morphogens" of the claimed invention refer specifically to the BMP subfamily of proteins, and include only a subset of all proteins encompassed by the broad term "TGF-beta superfamily," which includes many other non-BMP subfamily proteins such as Activin and TGF-beta (see review article by Heldin et al., Nature 390: 465, 1997 **Exhibit A**). Members of the BMP subfamily of proteins share significant sequence homology with one another, especially in the so-called C-terminal six or seven Cys skeletal structures. On the other hand, proteins belonging to the TGF-beta and Activin subfamilies share much less sequence homology, and use different receptors and intracellular signaling pathways (see **Exhibit A**, Table 1).

Similarly, the Office Action has misinterpreted the definition of "morphogen" as including growth factors such as Insulin Growth Factor (IGF) found in fetal calf serum (FCS) or fetal bovine serum (FBS). Obviously, morphogen of the instant invention does not include those growth factors in the first place. Even if it can be argued that certain morphogens may be present within FBS or FCS, for which the Examiner has provided no evidence to substantiate such a potential claim, Applicants submit that there are a myriad of factors within FBS or FCS, most of which remain uncharacterized even today. It cannot be assumed, without providing substantiating evidence, that Field teaches treating cells with morphogens rather than any other factors by growing cells in serum-containing media. Furthermore, contrary to the assertion of the Office Action, differentiation of cultured skeletal myoblast is induced by growing precursor cells in serum-poor media (such as those containing 2% horse serum, which is generally considered to contain less growth factors) as opposed to growing precursor cells in serum-rich media (such as those containing 20% FBS). Specifically, page 17 of Field describes that: "C2C12 myoblasts were derived from cultured explants of injured thigh muscle of C3H mice. When maintained in serum-rich media, the myoblasts proliferate rapidly and retain an undifferentiated phenotype. However, when cultured in serum-poor media myogenic differentiation is induced. TheC2C12 cells withdraw from the cell cycle and fuse, thereby forming multinucleated myotubes." Also in an earlier passage of the same example, on page 16, Field describes that: "For some studies,

myogenic differentiation was induced by culturing in DMEM supplemented with 2% horse serum and antibiotics." (emphasis added).

Finally, even if differentiation were achieved by growing cells in serum-poor media (as in Example 2 of Field), or by injecting TGF-beta1-expressing cells into myocardial tissue (as in Example 5), there is no evidence that these differentiated cells are indeed "functional myocardium" as required by the claimed invention, or as alleged by the Office Action.

In Example 2 of Field, C2C12 cells are of skeletal muscle origin (see above). The "Results" section indicates that "Myogenic differentiation is also induced, as evidenced by the appearance of numerous muscle-specific gene products" (page 17, lines 22-24), and "[t]he differentiated status of the grafted C2C12 cells was determined by immunohistological assay with an anti-myosin heavy chain antibody (MY-32). This antibody does not react with myoblasts nor with cardiac myosin heavy chain...As an additional control, hearts bearing AT-1 intra-cardiac grafts (see Example 1) were examined with the MY-32 antibody. No staining was observed, thereby ruling out the possibility that the signal seen in the C2C12 grafts was due to skeletal myosin heavy chain induction in host cardiomyocytes" (emphasis added). Thus, the injected C2C12 cells have differentiated from precursor cells, not into heart muscles (myocardium), but into skeletal muscles, since the MY-32 antibody (which does not stain heart muscle) stains these C2C12 grafts.

In Example 5, C2C12 cells expressing a MT-TGF-beta1 transgene were induced to differentiate by culturing in DMEM supplemented with 2% horse serum and antibiotics (page 29, 1st paragraph of Field), and were subsequently injected into myocardial tissues. There is no evidence that the differentiated cells are functional myocardium. In fact, Field does indicate that: "H and E analysis suggested that the C2(280) grafts were somewhat less differentiated as compared to those produced with unmodified C2C12 cells. This result was confirmed by immunohistologic analysis with a monoclonal antibody which recognizes skeletal myosin heavy chain" (1st paragraph of page 31, emphasis added). Therefore, TGF-beta1 expression appears to be *inhibiting* (rather than *promoting*) the differentiation of C2C12 cells injected into the myocardial tissues, and the differentiated C2C12 cells still resemble skeletal (rather than heart) muscles.

In conclusion, Field does not teach or suggest that myogenic precursor cells can differentiate into myocardium (in addition to differentiated skeletal muscles). Neither does Field teach or suggest that treating myogenic precursor cells with morphogen can induce proliferation or differentiation of precursor cells into functional myocardium. Therefore, Field cannot anticipate claim 1 and its dependent claims.

Similarly, Field does not anticipate claim 15, which is directed to a method of promoting proliferation of myogenic precursor cells or differentiation of myogenic precursor cells into functional myocardium, for the same reason presented above. In addition, Field is completely silent as to what constitutes a "morphogenically permissive environment."

Claim 20, directed to a therapeutic composition for promoting the repair or regeneration of mammalian myocardium; claim 24, directed to a method of culturing mammalian myogenic precursor cells; claim 28, directed to a method of inducing myogenic precursor cells, naturally competent to differentiate into skeletal or smooth muscle, to differentiate into cardio myocytes; and claim 29, directed to a method of producing replacement cardiomyocytes in a mammal in need thereof, are also not anticipated by Field for the same reasons as presented above.

Therefore, Applicants submit that all pending claims are novel in view of Field. Reconsideration and withdrawal of the rejection on grounds of 35 U.S.C. 102 is respectfully requested.

Claim rejections under 35 U.S.C. 103(a)

Claims 6, 7, 11, 12, 13, 14, and 17-19 are rejected under 35 U.S.C. 103(a) as being obvious over Field in view of Cohen.

Specifically, the Office Action alleges that Field does not teach a method of treating specific heart conditions, autologous muscle satellite cells, treatment steps, morphogen concentrations, and BMP proteins, and Cohen provides such information. Applicants respectfully disagree for the reasons which follow.

The claimed invention is partially based on the unexpected discovery that morphogen can promote the migration, proliferation and or differentiation of myogenic precursor cells (e.g.,

skeletal muscle satellite cells) into functional myocardium. Prior to the instant invention, it was believed that myocardial tissue lacks a sufficient number of myogenic precursor cells for adequate regeneration or repair of lost or damaged myocardial tissue. According to the specification, "[u]nlike skeletal muscle or smooth muscle, adult mammalian cardiac muscle has extremely limited powers of growth and regeneration...It is generally believed that there are no remaining myogenic precursor cells in adult mammalian myocardium and, therefore, lost or damaged myocardium is typically replaced by fibrotic or scar tissue, rather than new myocardium" (page 1, 2nd paragraph). "It has never previously been shown or suggested that treatment of myogenic precursor cells with the morphogens, morphogen inducers, agonists of morphogen receptors, or small molecule morphogenic activators is useful in promoting the proliferation and/or differentiation of myogenic precursor cells into new and functional myocardium in a morphogenically permissive environment. Nor has it previously been shown or suggested that morphogenically-treated myogenic precursor cells are useful in the treatment of lost or damaged mammalian myocardium" (page 5, 1st paragraph).

Pursuant to MPEP 2142, "[t]o establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)." Thus, to render the claimed invention obvious, all three criteria must be met.

First of all, Applicants submit that none of the cited references, either alone or in combination, teach or suggest that morphogen can be used to stimulate myogenic precursor cells to proliferate / differentiate into functional myocardium, either before, after, or simultaneously with the implantation of such precursor cells into a mammal.

As argued above, Field describes methods of delivering two types of muscle precursor cells, namely skeletal myoblast or cardiomyocytes (differentiated AT-1 cells or embryonic

cardiomyocytes), to heart tissues as grafts. Field also describe that such implanted grafts can serve as useful means of delivering recombinant proteins directly to the heart. However, Field is completely silent about morphogens. Consequently, it does not teach or suggest that morphogens can be used to treat these precursor cells, and to induce either their proliferation or differentiation into functional heart tissue (myocardium). Grafts in Field are either non-proliferating (page 18, 2nd paragraph of Field) differentiated (by growing in low-serum media rather than by being in contact with morphogen) skeletal muscle tissues (rather than myocardium) derived from skeletal muscle precursors (C2C12, see Examples 2 and 5 of Field), or partially proliferating myocardium which originates from *already differentiated* cardiomyocytes (AT-1 in Example 1 of Field) or embryonic cardiomyocytes (see Example 3, page 22, line 5 of Field), and which is not known to be induced to proliferate or differentiate by being in contact with any of the morphogens (See Examples 1, and 3 of Field). Therefore, neither of these two types of tissues is induced to proliferate or differentiate into functional myocardium by being in contact with a morphogen, as recited in claim 1. These defects are not corrected even in view of Cohen, since Cohen also does not teach or suggest that morphogen treatment can induce differentiation / proliferation of skeletal myoblast or embryonic cardiomyocytes into functional myocardium (see below).

Cohen describes in general terms that morphogens can be used to induce the growth of a variety of non-chondrogenic tissues in mammal. Although Cohen does cursorily mention skeletal muscles in a few places, there is no teaching or suggestion that those skeletal muscle precursor cells (such as satellite muscle cells) can be induced to differentiate into functional heart muscle (myocardium), let alone any teaching or suggestion regarding implantation of these cells into cardiac tissue of a mammal. In fact, Cohen teaches away from the claimed invention on page 6, lines 1-10, by reciting "In particular, these proteins are capable of inducing the proliferation of uncommitted progenitor cells, and inducing the differentiation of these stimulated progenitor cells in a tissue-specific manner under appropriate environmental conditions" (emphasis added). Therefore, a skilled artisan would reasonably conclude that contact with morphogen would only lead to differentiation of skeletal precursors into skeletal (rather than heart) tissues. Cohen is also completely silent regarding embryonic cardiomyocytes. Cohen does casually mention a few heart-related disease conditions such as cardiomyopathy (page 2, line 26 of Cohen), "damaged heart or blood vessel tissue", and "cardioembolic strokes" (page 6-7). However, there is no

description as to how morphogens can be used to treat these conditions. Without further elaboration, it cannot be assumed without providing evidence that Cohen specifically refers there to treating myocardial precursors with morphogen for the purpose of repairing damaged heart *muscle* – it is equally possible, based on the context these terms are used, that morphogens may be used to stimulate the repair process of other tissue types such as epithelial tissues in the blood vessel wall. Even if it can be interpreted that way, the instant specification has taught that there are very few, if any remaining myogenic precursor cells in adult mammalian myocardium. This is consistent with the established fact that the myocardium of adult mammal lacks regenerative capability (see page 119, the 3rd paragraph of Yoon et al., Texas Heart Institute J. 22: 119-125, 1995, submitted with the IDS on Dec. 14, 2001). Thus, one of ordinary skill in the art would expect that direct application / administration of morphogens to the damaged heart tissue without providing any precursor cells would likely fail to generate any new functional myocardium. Even if this indeed resulted in a partial repair of the damaged myocardium, it would still not fall within the scope of the present claims because it lacks the recited step of implanting precursor cells. These defects are not overcome by Field either, since Field also does not teach or suggest that morphogen treatment can induce differentiation / proliferation of any precursor cells into functional myocardium (see above).

Although Cohen also mentions *progenitor* cells, which, even if construed as "precursor cells" of the instant invention, are only used in a very broad sense (such as hematopoietic progenitor cells), and does not necessarily refer to muscle precursor cells. Simply because the broad term "progenitor cell" would include myogenic precursor cells does not mean that a skeletal myogenic precursor cell can differentiate into a heart muscle cell. In fact, Cohen never teach or suggest that a skeletal progenitor cell can "switch fate" after being in contact with a morphogen, and become a heart muscle cell useful for repairing heart tissues (rather than skeletal muscle tissues).

Thus, for the sake of argument, assuming a skilled artisan would be motivated to combine the teaching of Field and Cohen, the skilled artisan would still not have a reasonable expectation that morphogen treatment can induce the differentiation of muscle precursor cells into functional myocardium, because Cohen never teaches or suggests that skeletal muscle precursors can

differentiate into myocardium, and Field does not overcome this defect. Thus, the combined teaching still fails to satisfy the requirements of a *prima facie* case of obviousness.

Secondly, contrary to the Office Action's allegation, Applicants submit that a skilled artisan would have no motivation to combine the teachings of Field and Cohen in the first place. Field describes that either *differentiated* skeletal muscle cells (C2C12) or *bona fide* cardiomyocytes can be directly implanted into heart tissues and serve as grafts. Since grafts established from these implanted cells may already help to partially restore the function of damaged myocardium, treating these cells with morphogen really confers no additional benefits to the final outcome. In that respect, Field really describes a method of partially restoring myocardium function, but this method is different from what is presently claimed. Therefore, a skilled artisan practicing the Field method, even in view of Cohen, would have no motivation to additionally treat his cells with morphogen – after all, without knowing that morphogen treatment of skeletal precursor cells can lead to the differentiation of new myocardium, a skilled artisan would only conclude that treating skeletal precursor cells with morphogen is only an alternative way (as opposed to serum starvation) of inducing differentiation / proliferation into skeletal muscle cells.

Similarly, a skilled artisan would also have no motivation to incorporate the teaching of Field from the stand point of Cohen. The skilled artisan would know, based on Cohen, that morphogen can stimulate precursor cell proliferation / differentiation. Without knowing that skeletal precursor cells can differentiate into heart muscle cells after morphogen treatment, the skilled artisan could possibly consider treating skeletal precursor cells with morphogen for repairing skeletal muscle tissues, but would not think of treating skeletal precursor cells with morphogen for repairing heart muscle tissues. Thus, the teaching of Field is irrelevant for his purpose. On the other hand, the skilled artisan would understand that there are very few, if any remaining myogenic precursor cells in adult mammalian myocardium, thus direct morphogen treatment of damaged heart would likely be unsuccessful.

Therefore, based on the above argument, at least the *motivation to combine* and the *requirement that the combined teaching of the cited references must teach or suggest all the claim limitations*, two of the three criteria necessary to establish a *prima facie* case of

obviousness are not met. Applicants respectfully request reconsideration and withdrawal of rejections on grounds of 35 U.S.C. 103(a).

CONCLUSION

In view of the foregoing amendments and remarks, Applicants submit that the pending claims are in condition for allowance. Early and favorable reconsideration is respectfully solicited. The Examiner may address any questions raised by this submission to the undersigned at 617-951-7000. Should an extension of time be required, Applicants hereby petition for same and request that the extension fee and any other fee required for timely consideration of this submission be charged to **Deposit Account No. 18-1945.**

Respectfully Submitted,

Date: October 8, 2002

Yu Lu, Ph.D.
Reg. No. 50,306

Customer No: 28120
Docketing Specialist
Ropes & Gray
One International Place
Boston, MA 02110
Phone: 617-951-7000
Fax: 617-951-7050